DNA Procedures Introduction

Quality assurance measures ensure that laboratory tests yield results that accurately reflect the physical parameters they seek to characterize. As a part of their quality program, the DNA Units, which include, the DNA Casework Unit (DCU), DNA Support Unit (DSU), Federal DNA Database Unit (FDDU) and Scientific and Biometrics Analysis Unit DNA Group (SBAU), have developed standard operating procedures (SOPs) for the various testing methods used in the examination of evidence and database samples. Each laboratory SOP specifies the pertinent materials needed and the procedural steps to perform the test or tasks associated with the method to ensure the uniformity of each technique's performance over time and across scientists. This document provides guidance for the proper preparation of laboratory equipment and space, use of personal protective equipment, and general laboratory techniques as it applies to personnel in the DNA Units that work on forensic evidence samples, casework reference samples, and/or database samples.

1 Equipment

1.1 Supplies and equipment will be dedicated for pre- or post-amplification work areas and will not be moved from post- to pre-amplification work areas unless decontaminated. Post-amplification supplies and equipment will not be stored in rooms used for evidence examination or database sample processing unless decontaminated.

1.2 Pipettes dedicated to pre-amplification work areas must be used when performing pre-amplification activities (i.e., serological examination, sample collection, DNA extraction, qPCR, amplification set-up) and any other pre-amplification methodologies. A different dedicated set of pipettes must be used when performing post-amplification activities (i.e., sequencing, capillary electrophoresis set-up) and transferring liquid that potentially contains amplified DNA.

1.2.1 Handheld pipettes dedicated to pre-amplification activities must be irradiated using the interior ultraviolet (UV) light of the biological hood, a stratalinker, or equivalent for at least 5 minutes each workday before use. Also, they must be thoroughly decontaminated with a bleach solution and then disinfected/rinsed with 70% isopropyl alcohol each workday before use, as they become visibly soiled, and after their final use on a given workday. Pipettes dedicated to post-amplification activities must be cleaned weekly and as they become visibly soiled.

1.2.2 Sterile disposable pipette tips or transfer pipettes must be used when transferring liquid reagents or samples. A new pipette tip must be used when removing extract from a sample tube or when introducing reagent into a tube that contains extract. The tip must be discarded in the appropriate waste container after use.

1.2.3 To minimize the potential for pipetting inaccuracies, a pipette with a range larger than and closest to the target volume should be used. The pipette should be set to the desired volume

by initially dialing into the range of volumes larger than the target volume and then dialing back to the desired volume.

1.2.4 Robotic workstations that use fixed tips must be appropriately flushed with bleach and/or water between each sample and at the conclusion of a procedure.

1.3 New or clean forceps, scalpel blades, or scissors must be used for every sample. Tools must be appropriately discarded or decontaminated with a 10% bleach solution followed by 70% isopropyl alcohol between consecutive samples. Additionally, tools used for mitochondrial DNA (mtDNA) evidence examinations may be exposed to UV light before use. For SBAU cases where multiple samples of tape are taken from the same component of an item (e.g., tape from wires, tape from plastic), the same scalpel blade may be used consecutively without decontamination.

1.4 Biological hoods must be irradiated with their interior UV light for at least 5 minutes each workday before first use and after final use. A 15-minute exposure time is recommended for mtDNA processes.

1.5 For equipment that requires performance verification (i.e., performance check) prior to use, the results must be recorded in accordance with the LOM and DNA QA procedures.

2 Personal Protective Equipment

2.1 Disposable gloves must be used at all times during examination of evidence and sample processing. At a minimum, gloves must be changed if they become visibly soiled, torn, or when moving between separately packaged evidence items, with the exception of Sexual Assault Kit (SAK) swabs. Personnel are not required to change gloves between swabs collected from a single individual within a SAK.

2.1.1 To prevent transfer of biological material to laboratory surfaces that are not easily decontaminated (e.g., telephones, computer keyboards), used gloves should be removed prior to handling such laboratory devices. Double gloves may be worn to facilitate the removing and donning of outer gloves during those examination procedures in which notes are taken contemporaneously. Gloves must be changed and/or surfaces should be cleaned if inadvertent contact with a surface that may result in transfer of biological material is suspected (e.g., answering phone, scratching face).

2.1.2 When handling evidence items with potential latent fingerprint value, cloth gloves may be worn under the disposable gloves during processing. Nitrile gloves are preferred when processing items of potential latent fingerprint value.

2.1.3 Prior to leaving the laboratory area, used gloves must be properly discarded and personnel should wash their hands.

2.2 A laboratory coat must be worn during all pre-amplification processes. A separate laboratory coat must be worn when handling samples that potentially contain amplified DNA. Laboratory coats that are used in post-amplification laboratory space must not be worn into pre-amplification laboratory space. Laboratory coats should be placed in a laundry receptacle upon becoming visibly soiled. Laboratory coats must not be worn outside of designated laboratory space unless transporting evidence or samples.

2.3 Disposable face masks must be used at all times when handling evidentiary items or database samples, and when performing pre-amplification processes to minimize the potential for introduction of biological material by Laboratory personnel. Face masks must also be worn when preparing all pre-amplification reagents. Face masks do not need to be worn during post-PCR amplification processes. At a minimum, face masks must be changed if they become visibly soiled or torn.

2.4 Masks, gloves, bench paper, or tubes that are visibly soiled with biological material (e.g., blood, semen) must be placed into biological waste containers for disposal. Disposable items that do not show any visible biological staining may be discarded into regular waste containers.

2.5 Eye protection must be worn when performing laboratory tests and when handling reagents or chemicals.

3 Quality Assurance Safeguards

3.1 Pre-amplification work areas will be separated from post-amplification work areas. Amplified DNA is stored in the post-amplification work areas and must not be moved into the pre-amplification work areas.

3.2 All work surfaces in pre-amplification laboratory space must be decontaminated with a 10% bleach solution each workday before use, as they become visibly soiled, and after their final use on a given workday. All work surfaces within the post-amplification laboratory must be cleaned weekly, generally with detergent and water. Using bleach on the capillary electrophoresis instruments may interfere with fluorescence and should be avoided.

3.3 Disposable paper (e.g., bench paper, weigh paper, tissue paper) must be used when processing evidence items to ensure a clean working surface and to prevent the deposition of biological material on permanent work surfaces.

3.3.1 Disposable paper must be changed and appropriately discarded as it becomes visibly soiled or, at a minimum, before and after the completion of the examination of an individual item of evidence.

3.3.2 Evidence items that are packaged together (e.g., vaginal swabs, clothing items) may be processed on the same disposable paper, provided that the paper does not display visible soiling.

3.4 All evidence items or database samples under active examination must be kept separate from other items of evidence or database samples under examination by any other individual(s) working within a common laboratory space.

3.4.1 Evidence from only one case will be examined by an individual at a time and only one evidence package will be opened and collected for processing at any one time. The portion of the stain identified for analysis will be removed/collected from the item, placed into a corresponding labeled tube or envelope, and the item returned to the evidence packaging. This process will be sequentially repeated for each item within the case.

3.4.2 Only one database kit or sample will be open for processing (e.g., check in, punch) by an individual at any one time.

3.5 Reagents are stored separately from evidentiary and database samples. If the same storage area is used, at minimum, reagents must be placed on a different shelf and above evidentiary material or database samples.

3.6 Casework reagents will generally be dispensed into small aliquots to minimize the number of times the stock reagent is opened.

3.7 When necessary, reagent tubes should be thawed completely and vortexed briefly before use. As appropriate, the tubes can be quick-spun (approximately 2 seconds) to return all liquid to the bottom of the tube.

3.8 Examination of evidence or sample accessioning, DNA extraction, and amplification setup procedures may be performed in the same pre-amplification laboratory rooms if performed at separate times or at separate work stations.

3.8.1 DNA extraction procedures begin with the addition of the extraction reagents to the sample collected for DNA typing. All manual DNA extraction procedures must be conducted within a hood unless otherwise indicated.

3.8.2 DNA extraction steps in which phenol/chloroform/isoamyl alcohol (PCI) reagent is used must be performed in a chemical fume hood.

3.8.3 Manual DNA extraction and amplification setup procedures must be conducted within separate hoods or at separate times if performed in a common hood.

3.8.4 Automated DNA extraction and amplification setup procedures must be conducted on separate Robotic Workstations or at separate times if performed on a common Robotic Workstation.

3.9 While manually processing samples through a common procedural step, only one sample tube or reagent tube should be open at a time. Remaining sample tubes should remain closed.

3.10 All sample containing tubes that do not display a visible difference after the completion of a procedural step (e.g., color change, volume change, cutting introduction) must be physically moved or marked in a manner that distinguishes them from those on which that step has yet to be completed. This requirement will help to prevent the misloading or double-loading of samples during any procedural step that does not result in an evident physical change to a handled sample.

3.11 During the examination of an evidence item, notes (e.g., description of item, test result) must be recorded contemporaneously with conducting a procedure on or sample collection from that item. Such notes must be recorded in their final form (i.e., entered electronically). Multiple swabs from the same collection site and packaged together (e.g., vaginal swabs, oral swabs) may be processed together before being individually described in the final case records.

3.12 Extract tubes may be stored refrigerated or frozen. Upon retrieval from storage for subsequent examinations, samples should be brought to room temperature, vortexed (approximately 2 seconds), and quick spun (approximately 2 seconds).

3.12.1 For plate based samples, before removing an adhered cover (e.g., heat sealed cover) the plate should be centrifuged (approximately 30 seconds) to return all the liquid to the bottom of the wells.

3.12.2 Upon completion of testing, extract tubes may be stored at room temperature if the remaining extract is dried down. Instructions for using the Speed-Vac or Vacufuge are contained within the appropriate procedure (i.e., DNA 226) in the *DNA Procedures Manual.*

4 Safety

4.1 Refer to the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual* for important personal safety information prior to conducting these procedures.

4.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

4.3 Appropriate safety precautions and proper personal protective equipment will be used during laboratory procedures. Refer to Safety Data Sheets, *FBI Laboratory Safety Manual* and the applicable DNA Procedures for more detailed information. The following safety warnings are noted:

- Direct UV light can be harmful to eyes. UV-protective eyewear should be worn when observing UV lights during crosslinker QC checks.
- Agilent kit components contain dimethyl sulfoxide (DMSO). This dye binds to nucleic acids and will be treated as a potential mutagen.
- Ethyl alcohol is a hazardous material. Use only in a fume hood. Wear appropriate protective clothing and eyewear when handling; be careful not to expose face or hands to splashes.
- Formamide is a teratogen. Avoid inhalation, skin contact, or ingestion. Use nitrile gloves when handling. Dispose of unused portions in appropriate hazardous waste containers. Pregnant women must not handle Formamide.
- Hydrochloric Acid can be hazardous. Wear appropriate protective clothing and eyewear; be careful not to expose face or hands to splashes.
- Liquid nitrogen can be hazardous. Use cryogenic gloves, appropriate clothing and protective eyewear when handling liquid nitrogen. Be careful to avoid exposure to liquid nitrogen splashes.
- Phenol/Chloroform/Isoamyl Alcohol (PCIA) is an irritant and is toxic. Its use must be confined to a designated hood.
- Performance Optimized Polymer (i.e., POP-4, POP-6) is a chemical hazard and exposure may cause eye, skin and respiratory tract irritation.
- Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
- Sodium Dodecyl Sulfate (SDS) is an inhalation hazard. Wear a mask when working with powdered SDS.
- Sodium Hydroxide can be hazardous. Wear appropriate protective clothing and eyewear; be careful not to expose face or hands to splashes. A rapid release of heat can be produced when dissolving sodium hydroxide pellets.

5 References

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

FBI Laboratory Safety Manual

DNA Procedures Manual

Rev. #	Issue Date	History
1	06/01/17	Added BAU. 2.1 Changed to separately packaged and made allowance for SAK swabs 2.1.2 Nitrile gloves are preferred for handling latent items. 2.2 Lab coats may be worn outside lab space when transporting evidence. 3.2 Clarified bleach should not be used on CE equipment 3.7 Changed from centrifuged to vortexed 3.12.2 Extracts can be stored at room temp if dried. 5 Added references
2	06/01/21	Updated to SBAU. Changed methodology to tasks and editorial edits. 1.2.1: Added stratalinker or equivalent 1.3: Added exception for SBAU tape. 3.2: Reordered paragraph content and clarified masks required for all preamp reagents. 3.9: Clarified sample tube 3.12.2: Added Vacufuge.

Approval

Redact - Signatures on File

DNA Technical Leader Date: 06/01/2021

DCU Chief Date: 06/01/2021

DSU Chief Date: 06/01/2021

FDDU Chief Date: 06/01/2021

SBAU Chief Date: 06/01/2021

QA Approval

Quality Manager Date: 06/01/2021